bio-protocol Preprint

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CRISPR/Cas9-mediated targeting of TINF2 in RPE-1 cells

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Updated date: Jan 19, 2021

An abbreviated version of this protocol was published in eLIFE in Dec 2020

TINF2 is a haploinsufficient tumor suppressor that limits telomere length

DOI: 10.7554/eLife.61235

Detailed protocol

CRISPR/Cas9-mediated targeting of *TINF2* in RPE-1 cells

Materials

Details on the cell line, the sgRNA/Cas9 vector and the sequences of the sgRNA and donor oligonucleotides as well as PCR primers are indicated in the Methods section of our article.

Targeting

Please refer to our article for details on our targeting strategy.

Procedure

1. Clone desired sgRNAs into the Cas9-sgRNA expression vector. We used a vector that allows for co-expression of the sgRNA and Cas9 linked to mCherry via the T2A peptide (pU6-(BbsI)-Cbh-Cas9-T2a-mCherry (Chu et al., 2015)).
2. Order desired donor oligonucleotides (ssODN). We designed the ssODNs to have 50-60 nucleotide long homology arms on either side of the mutation(s) to be introduced.
3. Cultivate the required number of cells (hTERT-RPE1 p53-/- Rb-/-cells).
4. Trypsinize cells and count.
5. Add 7.5x 10^5 cells into a 15 ml conical tube.
6. Spin down cells (1000 rpm, 5 min and RT).
7. Aspirate medium and resuspend cells in 100 µl electroporation buffer (e.g. Nucleofector® Solution, Lonza)
8. Add 5 µg of high-quality DNA (for knock-in cell lines we used 1 µg of sgRNA/Cas9 expression vector / 4 µg ssODNs (1:1 mix of mutant and control), for TINF2+/- cells we used 5 µg sgRNA/Cas9 expression vector)
9. Mix well and transfer cells to electroporation cuvette.
10. Electroporate cells (e.g. Amaxa® Nucleofector®, Lonza, using the cell-appropriate electroporation protocol).
11. Transfer cells into 5 cm plate, pre-filled with warm cell culture medium and transfer cells to the incubator.
12. Change the culture medium 12-14 hours after the electroporation medium.
13. Perform two rounds of electroporation (48-72h apart) to improve efficiency.
14. 72h after the second electroporation select mCherry positive cells by single cell sorting and grow clones from single cells (sorted into 96 well plates).
15. Monitor cells for growth and expand cells for 2-3 weeks.
16. Expand a set of monoclonal lines and screen them appropriately.

How to cite:(Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Schmutz, I. (2021). CRISPR/Cas9-mediated targeting of TINF2 in RPE-1 cells. Bio-protocol Preprint. bio-protocol.org/prep759.
2. Schmutz, I., Mensenkamp, A. R., Takai, K. K., Haadsma, M., Spruijt, L., de Voer, R. M., Choo, S. S., Lorbeer, F. K., van Grinsven, E. J., Hockemeyer, D., Jongmans, M. C. and de Lange, T.(2020). *TINF2* is a haploinsufficient tumor suppressor that limits telomere length. eLIFE. DOI: 10.7554/eLife.61235